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PRODUCTION, IMMOBILIZATION AND INDUSTRIAL USES OF PENICILLIN G ACYLASE

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ABSTRACT

Penicillin Gacylase in one of the most important enzymes, it belonging to β-lactam antibiotics, first report on the enzyme penicillin acylase was in 1950 when they found in the mycelium of a *Penicillium* sp. The enzyme appeared to be a periplasmaticheterodimeric N-terminal serinehydrolase with a molecular mass of 86,183 Da, with a 23,817 Da (209 amino acids)α-subunit and a 62,366 Da (566 amino acids) β-subunit. This enzyme is capable of hydrolyzing penicillin G into phenyl acetic acid and 6-aminopenicillanic acid (6-APA) so this enzyme isthe starting material for the manufacture of penicillin derivatives, which are the most widely used β-lactam antibiotics. Both natural and semi-synthetic penicillins contain 6-aminopenicillanic acid.

Key Words: β-lactamantibiotics, Penicillin gacylase, Classification, Industrial uses.

INTRODUCTION

The β-lactam antibiotics

One of the most important groups of antibiotics, both historically and medically, is the β-lactam group. The β-lactam antibiotics include penicillins, cephalosporins, and cephamycins, all medically useful antibiotics. These antibiotics are called β- lactams because they contain the β- lactam ring system, a complex heterocyclic ring system. The β-lactam antibiotics act by inhibiting peptidoglycan synthesis in eubacterial cell walls. The target of these antibiotics is the transpeptidation reaction involved in the cross-linking step of peptidoglycan biosynthesis. Because this reaction is unique to bacteria, the β- lactam antibiotics have high specificity and relatively low toxicity.

Penicillin G, The first β-lactam antibiotic discovered, is active primarily against Gram-positive bacteria. Its action is restricted to Gram-positive bacteria primarily because Gram-negative bacteria are impermeable to the antibiotic. A vast number of new penicillins have been discovered, some of which are quite effective against Gram-negative bacteria. One of the most significant developments in the antibiotic field over the past several decades has been the discovery and development of these new penicillins.

The basic structure of the penicillins is 6-aminopenicillanic acid (6-APA)(Fig.1), which consists of a thiazolidine ring with a condensed β-lactam ring. The 6-APA carries a variable acyl moiety (side chain) in position 6. If the penicillin fermentation is carried out without addition of side-chain precursors, the natural penicillins are produced. The fermentation can be better controlled by adding to the liquid nutrient medium a side-chain precursor, so that only one disk penicillin is produced. Over 100 such biosynthetic penicillins have been produced in this way. In commercial processes, however, only penicillin G, penicillin V, and very limited amounts of penicillin O are produced (Fig.2)[1].

In order to produce the most useful penicillins, those with activity against Gram-negative bacteria, a combined fermentation/chemical approach is used which leads to the production of semi synthetic penicillins. The starting material for the production of such semi synthetic penicillins is penicillin G, which serves as the source of the 6-APA nucleus. Penicillin G is split either chemically or enzymatically (using penicillin acylase) and the 6-APA obtained is then coupled chemically to another side chain.

The production of penicillin and cephalosporin antibiotics is a multi-thousand tons industrial operation. Benzyl and

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phenoxymethylpenicillins (penicillin G and penicillin V respectively) are fungal fermentation products and the precursors to a wide range of semi-synthetic antibiotics (amoxicillin, ampicillin etc.). The chemical modification of the fermentation product is initiated by removal of the natural acyl group leaving the 6-aminopenicillanic acid (6-APA) as a penicillin nucleus. Alternative synthetic acyl groups can then be added to confer novel properties to the antibiotic such as resistance to stomach acid, a certain degree of penicillinase resistance or an extended range of antibiotic activity. The method of choice for the conversion to 6-APA at the industrial scale is the use of penicillin acylase. Penicillin acylases or amidases (EC 3.5.1.11) are a group of enzymes which can cleave the acyl chain of penicillins to yield 6-amino penicillinic acid (6-APA) and the corresponding organic acid, and in a number of cases the same enzyme can be used to direct the synthesis of the new antibiotic by the addition of the novel acyl group (Fig. 3)[2].

Penicillin G Acylase

Discovery and Occurrence

The first report on the enzyme penicillin acylase was in 1950 by **Sakaguchi and Murao** when they found in the mycelium of a *Penicillium sp*. the enzyme capable of hydrolyzing penicillin G into phenyl acetic acid and the unknown 6-APA (named "penicin")[3]. In the early years it was thought that penicillin G acylases were mainly produced by bacteria and penicillin V acylases mainly by molds. It is now well established that they are ubiquitous in bacteria, actinomycetes, fungi, and yeasts[4]. In *Escherichia coli* the gene encoding for penicillin acylase is located in a cluster of genes involving the metabolism of 4-hydroxyphenylacetic acid, where it is thought to have a function in the degradation of aromatics [5].

Classification

The penicillin acylasesare divided into three classes according to their substrate specificity. The penicillin V acylases (Type I) have a high affinity for phenoxyacetic acid derivatives whereas the penicillin G acylases (E.C. 3.5.1.11) (Type II) have a high affinity for phenyl acetic acid derivatives. The α-aminoacyl hydrolases (Type III) specifically hydrolyse α-aminoacyl β-lactam antibiotics. The penicillin acylases are classified as a new enzyme super family called the N-terminal nucleophile hydrolases or Ntn-hydrolases[6].

Structure and Catalytic Machinery

The crystal structure of *E. coli* penicillin G acylase was resolved by **Brannigan *et al.,*** in1995[6]. The enzyme appeared to be a periplasmaticheterodimeric N-terminal serinehydrolase with a molecular mass of 86,183 Da, with a 23,817 Da (209 amino acids)α-subunit and a 62,366 Da (566 amino acids) β-subunit. The enzyme is kidney-shaped (Fig. 4), approximate dimensions are 70×50×55 Å, with a deep cup-shaped depression leading to the active site. It has a single-amino-acid catalytic center, the β-chain N-terminal serine γ-hydroxyl. Penicillin acylase is believed to be activated by its own Ser β1 free α-amino group using a bridging water molecule, though more recently it was found that the reaction proceeds by a direct nucleophilic attack by the Ser β1O γ without the help of a bridging water molecule[7].

Structural insights in the catalytic machinery of *E. coli* penicillinacylase have been gained by resolving the crystal structures of several native and mutant enzyme-substrate complexes, e.g. with phenylacetic acid, phenylmethanesulfonyl fluoride, penicillin G sulfoxide, penicillin G, and D-α-methylphenylacetic acid [8].

The catalytically important amino acids can be divided into several classes. First one is the catalytically active nucleophile Ser β1. Second are the oxyanion hole formers Ala β69 and Asn β241 that stabilize the oxyanion transition-state intermediate. Third are the residues that play a role in enhancing the nucleophilicity of the Ser β1. These are Gln β23 (at 2.9 Å and 3.2 Å from Ser β1) and Asn β241 (3.0 Å from Ser β1). Fourth are the residues that are important for substrate binding. Where penicillin acylase has two substrate binding pockets, the most specific one, S1, is made up by mainly hydrophobic residues. The enclosed structure and largely hydrophobic character of the S1 pocket makes the enzyme very selective to the benzyl structure with some room for substitutions on the bridging C α and aromatic ring, e.g., -OH, -NH2, -CH3, -OCH3, -CN, -F, -Cl, -Br. Ring structures other than phenyl are also accepted, e.g., pyridyl, thiophene, thiazole, 1H-tetrazole, and furanyl. The principle residues that enclose the S1 pocket are Met α142, Phe α146, Phe β24, Phe β57, Trp β154, Ile β177 with Ser β67 at the closed end. The residues that complete the enclosed structure are Pro β22, Gln β23, Val β56, Thr β68, Phe β71, Leu β253, and Phe β256. The S2 pocket, or (β-lactam) nucleophile-binding pocket, is in reality the bottom of the cup-shaped depression mentioned before and therefore makes for a very broadsubstrate specificity of this pocket. In contrast to the S1 pocket, the S2 pocket is enantioselective and can therefore be used for e.g. amine resolution [9]. It is formed by Arg α145, Phe α146, Phe β71, and Arg β263.

The catalytic mechanism of *E. coli* penicillin G acylase catalyzed amide hydrolysis isshown in figure 5. The first step in penicillin acylase catalysis is the nucleophilic attack of the active Ser β1: O γ hydroxyl on the electrophilic carbonyl carbon of the amide substrate. The tetrahedral oxyanion transition-state intermediate is stabilized by hydrogen bonding with two amino acids in the oxyanion hole (Ala β69: N and Asn β241: N δ2). Next, the covalent acyl-enzyme intermediate is formed when the carbonyl group is restored under release of the product P (e.g., 6-APA, NH3, CH3OH in the case

of penicillin G, D-phenylglycine amide (D-PGA) or methyl ester (D-PGM), resp.). In the following step the acyl moiety is transferred to either a water molecule (hydrolysis) or to an amine nucleophile (e.g., a β-lactam nucleus or any other amine) in which case an amide bond is formed (synthesis).

Penicillin acylases (PGAs) family is divided into classes based on their substrate preference[7]. It has been suggested that PGA in *Escherichia coli* may function during the free-living mode of the bacterium to degrade phenylacetylated compounds generating phenyl acetic acid (PAA), which may be utilized by the organism as a carbon source [10]. Penicillin acylases are valuable pharmaceutical enzymes and are used in the synthesis of many semi-synthetic penicillin derivatives and in hydrolysis of β-lactam antibiotics[11]. Penicillin G acylase catalyzes the conversion of benzyl penicillin, via hydrolysis of the amide bond in the benzyl penicillin side chain, to release phenyl acetic acid and 6-aminopenicillanic acid (6-APA), the latter being the important precursor utilized in industrial synthesis of semi-synthetic penicillins[10].

In nature, PGA is initially produced as a single-chain precursor in the cytoplasm of *Escherichia coli*, and after removal of several polypeptides, the enzyme reaches a mature state in the periplasm[10]. The mature enzyme is a heterodimer of a small α-unit (209 residues) and a large β-unit (557 residues. PGA is characterized as an N-terminal-nucleophile (Ntn)hydrolase; the Ntn super family is comprised of enzymes that share a common fold around the active site and that contain a catalytic serine, cysteine, or threonine at the N-terminal position [8]. The two chains form a pyramidal shaped structure, and in the middle of the pyramid resides the hydrophobic active site. The active site of PGA is comprised of several hydrophobic amino acid residues, making it very specific for the phenyl acetyl group of PG [11].

Since the discovery of penicillin acylases (PGAs) in the 1950's, they have been incorporated throughout the pharmaceutical industry and exploited for industrial purposes. Penicillin acylases are widely distributed among many microorganisms, including bacteria, actinomycetes, yeasts, and fungi[12].

Penicillin G acylase (EC 3.5.1.11) is commonly isolated from *Escherichia coli* strain W (ATCC 1105) for its use in pharmaceuticals [13]. An immobilized form is used commercially to cleave benzyl penicillin (PG) via hydrolysis of the side-chain amide bond, to yield phenyl acetic acid (PAA) and 6-aminopenicillanic acid (6-APA), the latter of which is an important precursor for the synthesis of several semi-synthetic, β-lactam antibiotics (Fig. 6)[14,15]. The combined industrial uses of penicillin G acylase and penicillin V acylase results in the annual production of 9000 tons of 6-APA[16]. Though the mechanism and function of PGA for commercial use is widely understood, much uncertainty surrounds the *In Vivo* function and mechanism of PGA. The *In Vivo* expression of PGA has been observed to be regulated by both temperature and phenyl acetic acid, leading to the putative role that it is employed during the free-living mode of the organism to generate a carbon source, by degrading phenyl acetylated compounds to generate phenyl acetic acid[10].

Penicillin acylases belong to a super family of enzymes, N-terminal nucleophile hydrolases (Ntn-hydrolases), which are generally synthesized as precursor proteins and undergo a post-translational autocatalytic process to generate the mature protein with an active catalytic center at the new N-terminus [17].The mature, active enzyme is formed by removing a linker peptide (30-50 amino acids) in the proenzyme, resulting in a heterodimer (A and B Chains) with a free N-terminal nucleophile, either a serine, cysteine, or threonine[18]. In the case of mature PGA, the N-terminal nucleophile is a serine residue (Ser1) on the carboxyl terminal of the B-chain. This super family of enzymes shares not only a similar generation process, but also a similar tertiary structure[18].

The characteristic fold of Ntn-hydrolases consists of a four layered catalytically active αββα-core that is comprised of two antiparallel β-sheets packed against one another and covered by a layer of antiparallel α-helices on one side (Fig.7)[19, 20].

Penicillin G acylase(PGA) is synthesized in the cytoplasm of *E. coli* as a single chain precursor. The precursor contains a signal sequence of 26 amino acids and a spacer peptide composed of 54 amino acids (Fig.8a)[17]. The signal sequence is used to direct the translocation of the proenzyme to the periplasm, while the spacer sequence, between chains α and β blocks the active site and is also thought to influence the final folding of the protein [17].The signal sequence and the linker peptide are removed via an autocatalytic process, resulting in the mature form of the enzyme in the periplasm of *E. coli*[10].

The mature, active *E. coli* PGA enzyme is an 86 KD heterodimer, with chains α (209 amino acids) and β (557 amino acids) closely intertwined and held together by non-covalent forces (Fig.8b)[10].

The crystalline structure of PGA isolated from *E. coli* W and grown in presence of ethylene glycol has been determined to a resolution of 1.3 Å. The enzyme forms a pyramidal structure with a centrally located deep depression, at the bottom of which is the active site[17].The binding pocket is lined with hydrophobic residues from the α and β subunits, defining its specificity for the phenyl acetyl group of penicillin G [11].

Conditions for production, extraction and immobilization of PGA

The most widely used organism is *E. coli*, containing either the endogenous gene or a heterologous gene. Fermentations

are performed in up to 250m^3 fed batch, stirred fermenters. Seed cultures are grown at 37 ^{o}C for a prescribed time; usually a pH change indicates optimal time for transfer, then up to 10% seed can be used to inoculate the production fermenter. After an initial growth period, the temperature is reduced and carbon feeding is initiated. This is the trigger for PGA production. The fermentation is continued until maximum activity is achieved and then the fermentation is harvested[2].

The level of dissolved oxygen is required to be maintained at 15% or higher to avoid repression of PGA. In addition, since glucose is a known repressor of PGA activity when used as carbon source its rate of supply has to be limited. Alternative carbon sources such as sucrose or glycerol are commonly used. The pH of the culture needs to be maintained in the range 6.8 to 7.1 for optimal PGA production. Likewise, the production temperature has to be maintained below 30 ^{o}C. Since the expression of the *pac* gene can be differentially modulated depending on the exact nature of the plasmid constructed, diverse expression inducers can be used to switch on the *pac* gene fused to a particular upstream sequence. Examples include galactose[21], and isopropyl-beta-D-thiogalactopyranoside (IPTG) to induce the *lac* promoter, and rhamnose to switch on the *rha*BAD promoter.

Since the active PGA enzyme is localized in the periplasmic space in *E. coli* this immediately affords a purification step as less than 5% of cellular proteins are found in this compartment[2]. The periplasmic fraction can be separated from the cytoplasmic fraction using selective permeabilization. Cell permeabilization by osmotic shock in combination with EDTA has been reported to yield 70% protein without affecting cell viability. Polar organic solvents and aqueous solutions of ionic and non-ionic detergents have been used to permeabilize cells. For example, on a laboratory scale, treatment with guanidine/EDTA has been reported to give a 93% yield with a 25-fold purification. Solvents with dielectric constants below five and having high hydrophobicity released the most active PGA protein. The production of reverse micelles using AOT in water/hexane resulted in the selective release of the enzyme without cell breakage[22].More specific means of purification of PGA have been demonstrated. It is achieved that greater than 65–fold purification by immobilized metal affinity chromatography (IMAC). The advantage of this technology is that it can be scaled up and the matrices are suitable for sanitization.

Whilst the permeabilization methods outlined above can be applied to small scale cultures, the release of PGA from industrial fermentations poses greater problems.

To this end the large scale release of PGA usually involves physical disruption followed by partial purification. After fermentation cells are harvested by centrifugation or settled with flocculants. The concentrated cells are then homogenized and cell debris removed. The extract is then purified by the method of choice. The most general means of purification is chromatography and/or ammonium sulphate precipitation. A typical method may involve a pH shift and heating to selectively denature sensitive proteins leaving PGA active in the mix.

The use of two-phase affinity partitioning as a means of protein purification is well documented. A polyethylene glycol (PEG) derivative and salt system has been shown to be effective in the purification of PGA[23]. The PEG derivative is selected on the basis of its expected interaction through hydrophobic, electrostatic and biospecific effects. In most cases the ligand has a structure analogous to the penicillin G substrate of the enzyme. Thus benzoateand phenylacetamide derivatives are useful ligands. The salt phase usually contains sodium sulphate or sodium citrate.

Thermostability can be an important feature for enzymes used in industrial processes as higher temperatures can be used to enhance reaction rates, shift thermodynamic equilibria, increase reactant solubility and decrease the reaction viscosity. The catalytic performance of PGA increases at temperatures between 25^{o}C and 50^{o}C. However, the enzyme shows poor stability at temperatures above 35^{o}C and therefore an effective means of providing thermalstability is highly desirable. Many methods of PGA thermo stabilization have been studied. Thermal unfolding of penicillin acylases has been linked to their conformational mobility in water. This mobility can be reduced by diminishing the amount of free water which can be achieved by the addition of stabilizers such as polyolcompounds[24], bisimidoesters[25], neutral salts or proteins. The stability of PGA was improved by up to 180% by the addition of trehalose after thirty minutes incubation at 60 ^{o}C[26].

In order to be useful as a biocatalyst the PGA enzyme preparation has to be active, robust and re-usable. One of the most effective ways to enhance stability for many enzymes is to immobilize the enzyme onto a solid support. In addition, immobilization may allow re-use of the catalyst and thus increase cost effectiveness. A number of immobilization methods have been used in this context[27]. Each method shows superiority to the more traditional use of free cells, extracts or even immobilized whole cells. Immobilized enzyme preparations attain higher activity and specificity and show better control of contamination[28].

The most common immobilization methods include cross-linking and covalent attachment. Glutaraldehyde is the usual cross-linking agent used. A 15-foldimprovement in thermostability was apparent after cross-linking with dimethyladipimate. The use of different physical forms of chitosan (powder, particles or beads) to immobilize PGA either by adsorption followed by reticulation with glutaraldehyde or

by direct cross-linking to the matrix pretreated with glutaraldehyde has been reported[29].

Also it is used double entrapment methodology for the immobilization of PGA on agar-polyacrylamide resins. A highest specific activity of 322U/g wasobtained by covalent binding of PGA onto vinyl copolymers. At present, commercial manufacturers such as Resindion supply epoxy group resinssuch as Sepabeadsfor use as immobilization matrices for enzymes including PGA. Sepabeads are porous spherical beads with outstanding mechanical stabilityand extensive cross-linking. Multipoint covalent immobilization of PGA from*K. citrophila* stabilized the enzyme 10,000-fold compared to the soluble enzyme from *E. coli*.

A further means of PGA enzyme stabilization is afforded by the production of cross-linked enzyme crystals or CLECs. This approach is unique in that it results in both stabilization and immobilization without activity dilution. The protein matrix is both the catalyst and the support. The crystals are produced by stepwise crystallization of the purified enzyme followed by molecular cross-linking to preserve the structure, resulting in a biocatalyst which is extremely stable to both temperature and organic solvents. The stabilization is a consequence of both polar and hydrophobic interactions. The PGA CLEC has been commercialized for both hydrolytic and synthetic activity use, and retains activity over more than 1000 batches[30]. Another novel preparation called a cross-linked enzyme aggregate (CLEA) has also been reported[31]**.** CLEAs are prepared by slowly adding a precipitant such as ammonium sulphate to the enzyme at low temperature. The aggregated enzyme is subsequently linked with glutaraldehyde and is then available for use as a biocatalyst. The CLEA canbe used in aqueous media in both forward and reverse reactions. Unlike the CLEC preparation, the CLEA enzyme need not be purified to near homogeneity.

Industrial uses of PGA

The most widespread use of PGAs is in the production of 6-APA from bothPen G and Pen V. Immobilized PGA enzymes mainly from *E. coli, B. megaterium and A. faecalis* are available from a number of commercial suppliers. Reactionsare carried out at >5,000L scale under controlled conditions, the pH being eithercontrolled at approximately 8.0, or slowly ramped from 7.0 to 8.5, depending upon thecatalyst, as high as 8.5. Exposure to high temperature (>30 °C) and pH (>8.0) isminimized to reduce inactivation of the enzyme and retain high product yield of the otherwise relatively unstable 6-APA. The use of PGA in large scale production of semi synthetic penicillins and cephalosporins is also widespread. These processes are focused on the condensation of an appropriate D-amino acid derivative with a β-lactam nucleus in a PGA catalyzed reaction. This involves the direct acylation of nucleophiles such as 6-APA or 7-ADCA with free acids at low (<7.0)pH.

Production of 6-APA by immobilized PGA

Enzymatic production from benzyl penicillin (Pen G)of 6-aminopenicillanic acid (6-APA) represents one of the few commercially established enzymatic processes in the pharmaceutical industry. The enzyme employed, penicillinG acylase (PGA), is immobilized on various solid supports using conventional immobilization techniques. The performance of immobilized penicillin Gacylase (IMPGA) is determined by the type of reactor used[32].

This Enzyme isthe starting material for the manufacture of penicillin derivatives, which are the most widely used β-lactam antibiotics. Both natural and semi-synthetic penicillins contain 6-aminopenicillanic acid. Different penicillin types differ in their attached side chains. Semi-synthetic penicillin may be produced by enzymatic removal at the side chain of native penicillins with subsequent attachment of a novel side chain to the resultant 6-aminopenicillanic acid core[33]**.**

Kinetically controlled synthesis involves an acyl group transfer reaction in which activated acids, esters or amides are used as the acylating agents. The yield of this type of reaction is dependant upon three different reactions carried out by the enzyme: 1) the synthesis of the β-lactam compound, 2) the hydrolysis of the activated acyl donor, and 3) the hydrolysis of the product. There are many ways to optimize such a reaction including optimizing pH[34]**,** addition of suitable solvents[35] and the use of high concentrations of acyl donor and nucleus. Using such controlled strategies a number of different antibiotics are produced including amongst others the high volume antibiotic products amoxicillin[36], ampicillin[37], cephalexin [38]and cefazolin[39]**.**

An alternative use of PGA is in peptide synthesis. The acylase can be used for the protection and deprotection of amino groups of amino acids by direct enzymatic synthesis and acyl group transfer reactions. For example PGA has been used as a biocatalyst in the synthesis of the sweetener aspartame, and further use has been in the preparation of D-phenyl dipeptides whose esters readily undergo ring closure to the corresponding diketopiperazines. Such peptides are used as food additives and as synthons for fungicidal, antiviral and anti-allergenic compounds. In addition, PGA can hydrolyze phenyl acetyl derivatives of a number of peptides and resolve enantiomers of some organiccompounds[40]**.**

The commercial viability of any enzyme depends on its operational stability and reusability. Enzymes in free form are thermolabile and cannot be reused, owing to their loss during downstream processing and purification of the product. Immobilization is the most important technique for stabilizing enzyme activity and enhancing its operational life. Immobilization does not necessarily enhance the enzyme's stability, but this can be achieved by different modes of the immo-

bilization matrix system. PGA is one of the most common commercially significant examples of enzyme reusability [41].

In the past, a number of immobilization systems have been patented and commercialized for PGA production[42]. Whole cells are being entrapped with a certain ratio of polyethylimine and glutaraldehyde and used as a catalyst for antibiotics' conversion into intermediates. In liquid, PGA has been immobilized on a number of novel carriers, such as ethylene glycol dimethacrylate, Eupergit C, alumina beads, nylon fibers, silica support, zerogel, sepa beads, glycoxylagarose, and a number of anionic exchangers. Hydrophobic interaction chromatography that concentrates and purifies the enzyme in a single-step process was explored by fabricating macroporous weak cation-exchange methacrylate polymers to immobilize PGA[43].

CONCLUSION

One of the most important β-lactam antibiotics is Penicillin Gacylase which is active against Gram positive bacteria. This Enzyme is the starting material for the manufacture of penicillin derivatives, which are the most widely used β-lactam antibiotics. Penicillin acylases or amidases (EC 3.5.1.11) can cleave the acyl chain of penicillin to yield phenyl acetic acid, which is used as a carbon source, and 6- amino penicillinic acid, back bone of β-lactam antibiotics. In order to be useful as a biocatalyst, the Penicillin G acylase need to be stable and used many times. One of the most effective ways to enhance stability for many enzymes is to immobilize the enzyme onto a solid support. In addition, immobilization may allow re-use of the catalyst and thus increase cost effectiveness.

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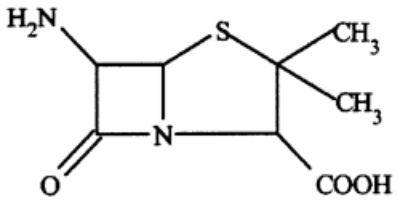
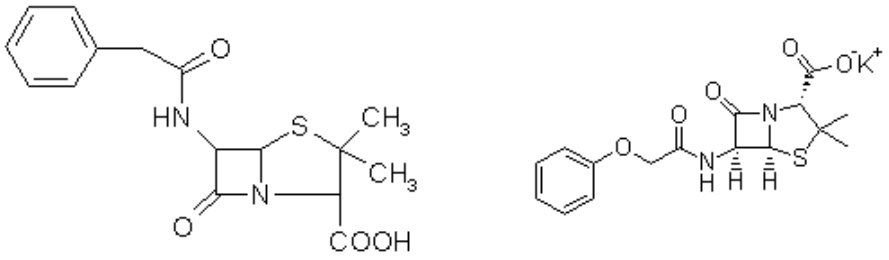


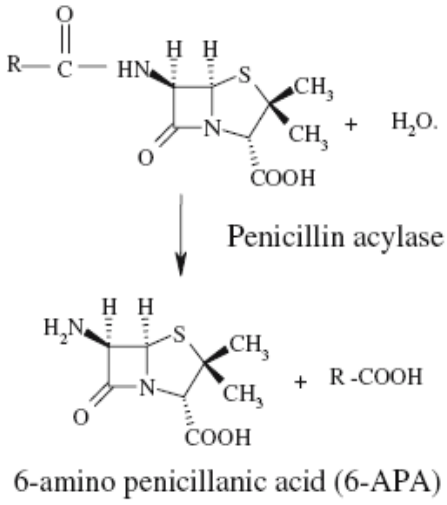
Figure 1: 6-aminopenicillanic acid



Penicillin G **Penicillin V Potassium salt**

Figure 2: Different types of penicillin

Antibiotic	R
Penicillin G	$-CH_2-$
Penicillin V	$-O-CH_2-$
Ampicillin	$-CH(NH_2)-$
Penicillin K	$CH_3-(CH_2)_4-$
Penicillin dihydro F	$CH_3-(CH_3)_4-$
Penicillin F	$CH_3-CH_2-CH=CH-CH_3-$



6-amino penicillanic acid (6-APA)

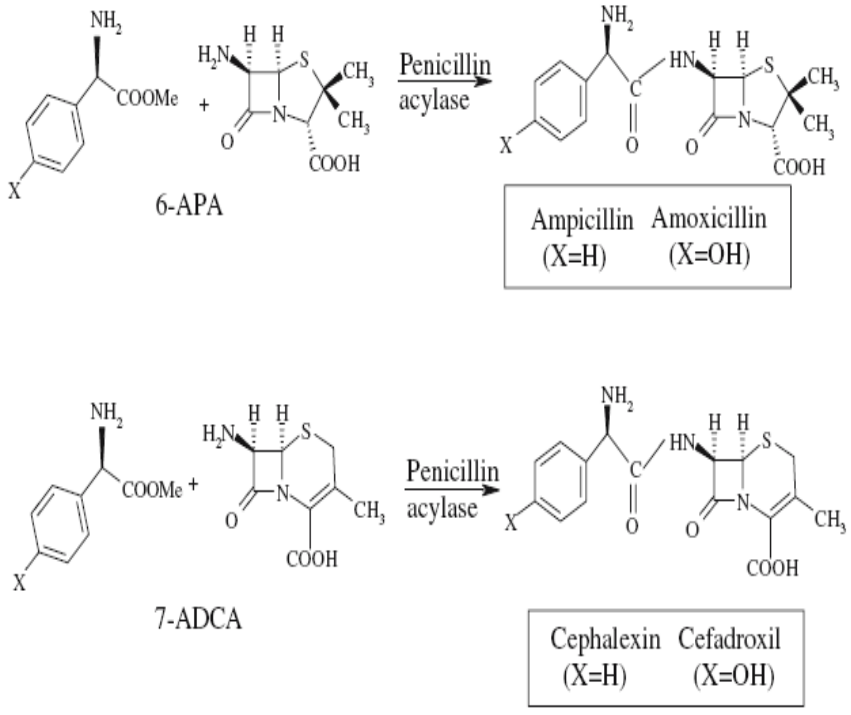


Figure 3: Reactions of Penicillin Acylase

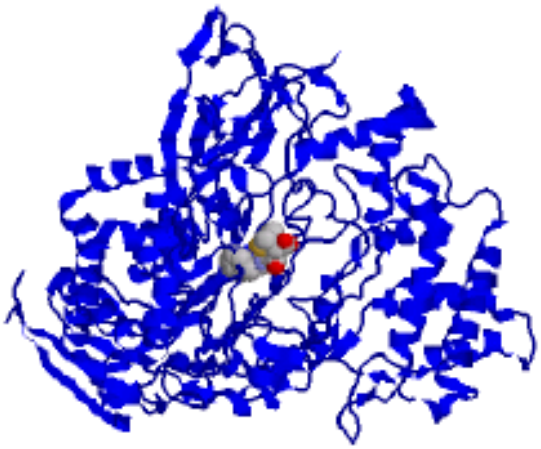


Figure 4: Penicillin G Acylasecomplexed with Penicillin G [7].

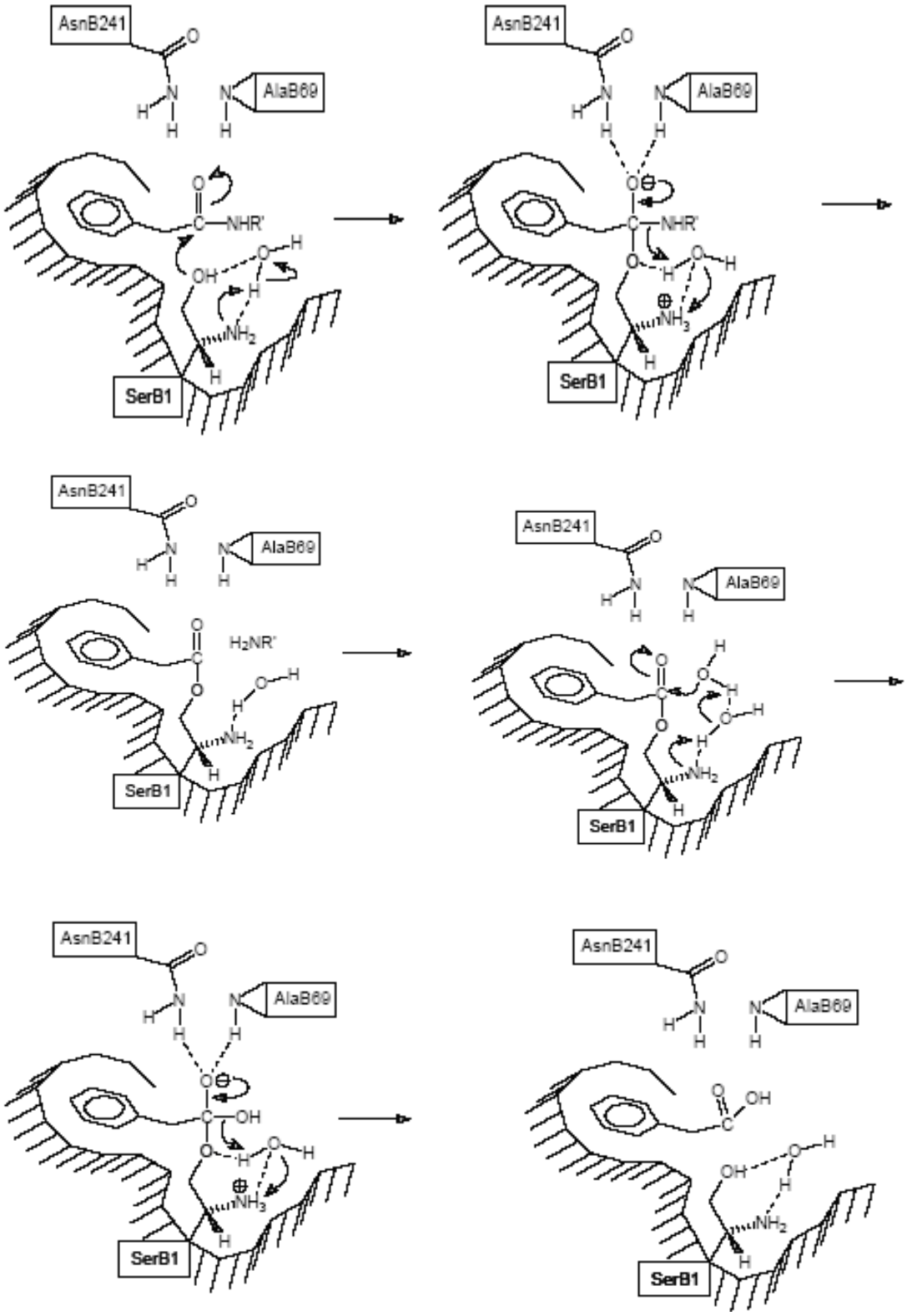


Figure 5: Catalytic mechanism of penicillin G acylase catalyzed amide hydrolysis[9].

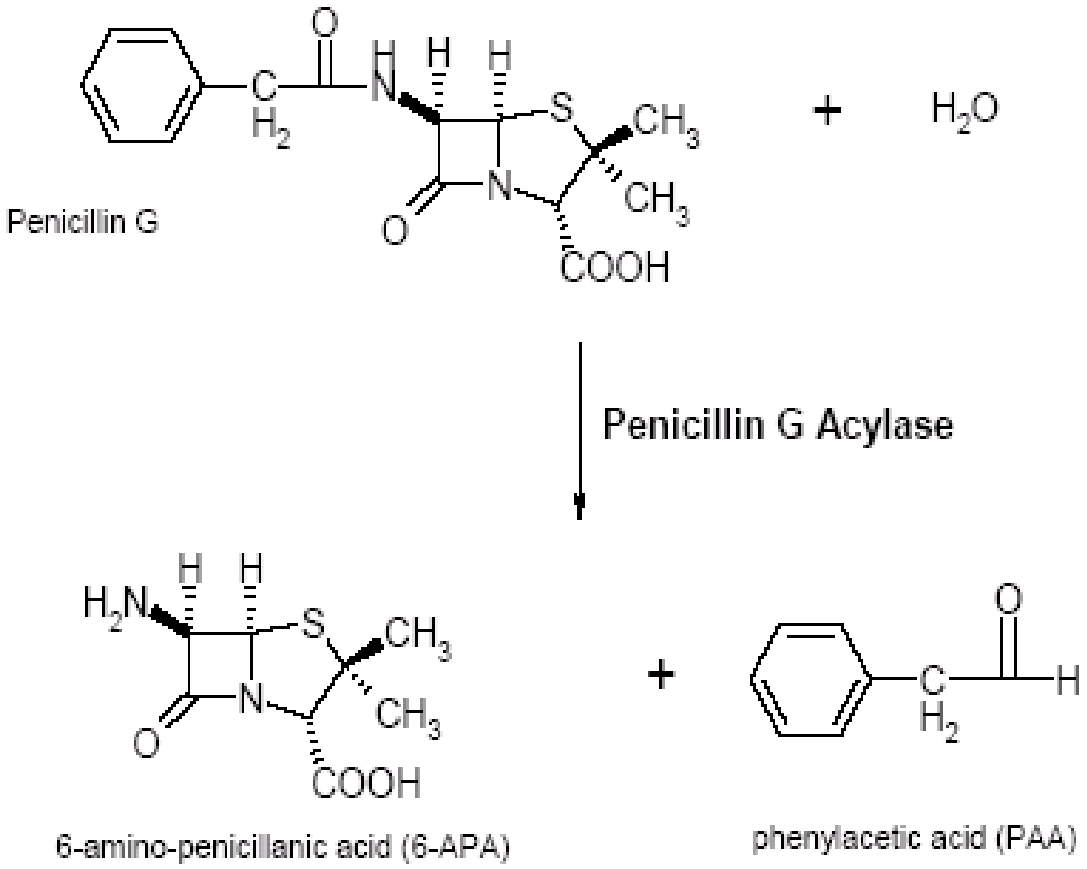


Figure 6: Reaction catalyzed by penicillin G acylase

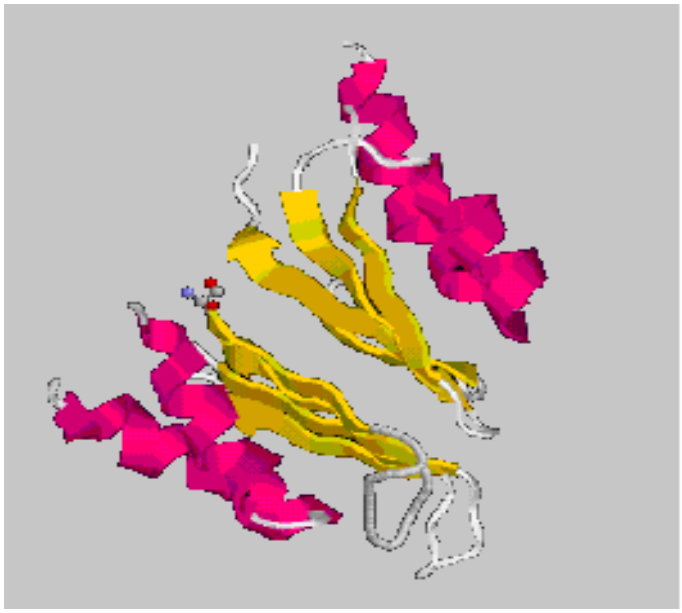


Figure 7: Structure of the characteristic N-terminal nucleophile -fold with the active site serine displayed in ball-and-stick.[7]

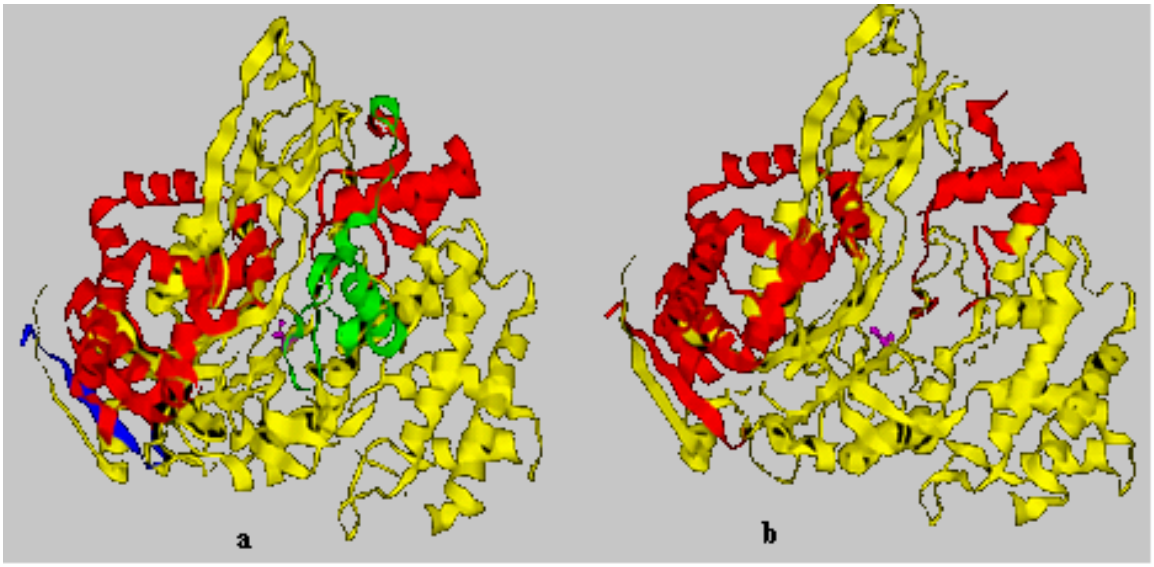


Figure 8: (a) The secondary structure of the precursor to PGA. The signal sequence is shown in blue,linker sequence in green (blocking the active site), and the sequences of amino acids that will form the α and β chains after processing are shown (red and yellow, respectively).
(b) Structure of mature PGA showing secondary structural units by chain (red=Chain α, yellow=Chain β). The active site residue, Ser1: B is shown in magenta in both structures[10].